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Simple and efficient 'panning' of transfected cell populations expressing a cell surface marker

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▼Preparative separation of cell populations on the basis of characteristics of cell surface markers represents an important technique for numerous research applications in biology and medicine. Increasingly, this approach is being adopted for the purification of mammalian cells transduced with DNA, as a preferred alternative to drug selection in studies on ectopic modulation of gene function. By using antibodies to surface markers encoded by co-transfected genes [e.g. CD2 (Ref. 1) or immunogloblins], several methods such as fluorescence activated cell sorting (FACS) immuno-magnetic bead separation and simple 'panning' have been used to facilitate the purification of the target, transduced cell population from what is often a vast excess of non-transduced cells Of these methods, panning is by far the simplest and least expensive (Ref. 2) but is generally considered to be too crude for most applications. We report here on some simple modifications to positive selection of antigen-marked cell populations by panning that dramatically enhances the performance of this procedure.

Petri dishes (e.g. 3.5 cm diameter Sterilin, bacteriological grade) are pre-coated overnight at 4°C with the appropriate antibody [10 μg/ml in phosphate buffered saline (PBS)], washed several times with PBS and treated with bovine serum albumin blocking solution (0.2% in PBS) for 1 h at room temperature before application of the cell suspension (approximately 2 X 10^6 cells in 4 ml of standard cell growth medium per dish). Instead of relying on gravity to sediment the cells as in conventional panning, the Petri dishes are centrifuged for 10 min at 500 rev/min in a bench centrifuge swing-out rotor (e.g. MSE Mistral 2000) at room temperature, supported on the top of plastic, multi-well tube adaptors. By compacting the cells onto the antibody-coated surface in this way, the antigen-mediated bonding of the positive cell population is enhanced, whilst antigen-negative cells are still readily detachable from the surface, provided higher centrifugation speeds are avoided. In conventional panning, this latter cell population is removed through several 'washing' stages (Ref. 2), a rather inefficient procedure which can also result in significant and unacceptable loss of the target cell population. To overcome this problem, we have developed a procedure for quantitative removal of unbound cells by gravity sedimentation which can be performed under aseptic conditions in a laminar flow cabinet. Following the centrifugation step above, the level of liquid in the Petri dish is increased by very gently pipetting on fresh medium until the surface meniscus is raised just above the top of the dish. A Petri dish lid of somewhat larger dimension (e.g. 6 cm) is then gently laid on the top, eliminating any air bubbles (a small overflow of medium at this stage is inconsequential). The whole assemblage, which is now robustly held together by suction, is swiftly inverted and unbound cells allowed to sediment for around 15 min at room temperature. The Petri dish, still in the inverted orientation, is then briskly 'peeled off', allowing the medium containing unbound cells to collect in the lid below whilst retaining the antibody-bound cells in the dish to be recovered by re-suspending in fresh medium.

In the working example illustrated (Fig. 1), human Daudi B lymphoblastoid cells were co-electroporated with DNA constructs encoding a truncated rat CD2 surface antigen to facilitate purification (Ref. 1), a luciferase reporter to quantitate recovery (Ref. 3) and a β-galactosidase (*lacZ*) marker to monitor purification (Ref. 4). From a starting population comprising 5–10% *lacZ*-positive cells (Fig. 1a), 65% of the luciferase activity was recovered as a CD2-positive population containing approximately 80% *lacZ*-positive cells (Fig. 1b).

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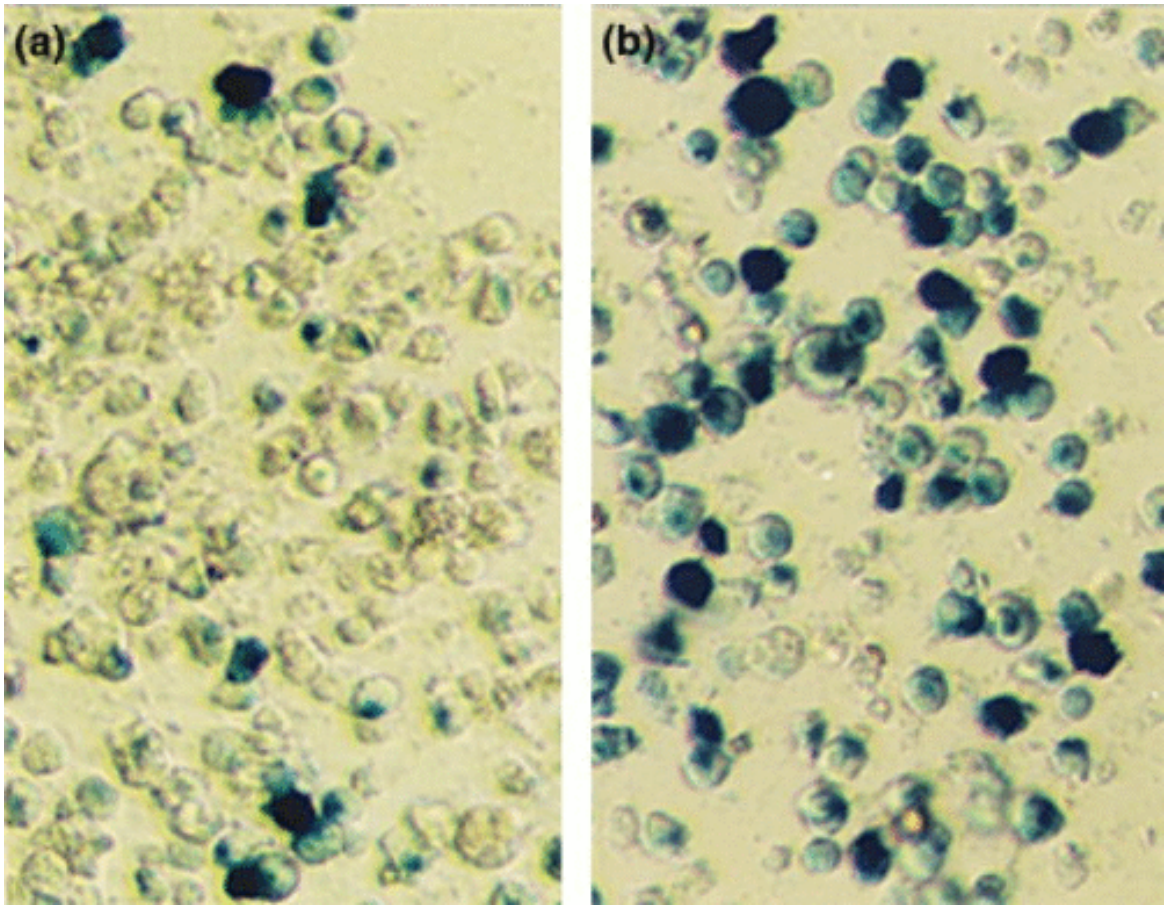

FIGURE 1. Purification of *lacZ*-transfected cells using a CD2 surface marker. Approximately 10^7 Daudi B lymphoblastoid cells in 350 μl of RPMI-10% fetal calf serum were co-electrophoresed with 2.5 μg of each of the following DNA constructs; pRCD2- 2 (Ref. 1) encoding a truncated rat CD2 antigen, each of the plasmids pUHGI5-1 (Ref. 3) and pUHC13-3 (Ref. 3) encoding the tetracycline-responsive transactivator (tTA) and a tTA-responsive luciferase reporter, respectively, and CMV β-gal (Ref. 4) encoding the *Escherichia coli* β-galactosidase gene, using a Biorad electroporator (set at 950 μF, 280 V). After 24 h, 2 X 10^6 cells were subjected to purification using immobilized anti-rat CD2 antibody (Serotec) as described in the text. The photographs show X-gal-staining (Ref. 4) of β-galactosidase-positive cells (a) in the unfractionated population and (b) following purification of CD2-positive cells.

Although we developed this procedure for the specific application of purifying transiently transfected cells, the method would be equally applicable to any circumstance requiring preparative purification of specific cell populations (including stable transfectants) on the basis of surface antigen characteristics. Because the operation is completed within 30 min, excellent cell viability is preserved, no specialized expensive equipment is required and the method can be adapted for use on any scale.

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Products Used

anti-rat CD2 antibody: anti-rat CD2 antibody from Serotec Ltd